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ORIGINAL PAPER

Molecular and comparative mapping of genes governing spike compactness from wild emmer wheat

Justin D. Faris · Zengcui Zhang · David F. Garvin · Steven S. Xu

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Abstract The development and morphology of the wheat spike is important because the spike is where reproduction occurs and it holds the grains until harvest. Therefore, genes that influence spike morphology are of interest from both theoretical and practical stand points. When substituted for the native chromosome 2A in the tetraploid Langdon (LDN) durum wheat background, the *Triticum turgidum* ssp. *dicoccoides* chromosome 2A from accession IsraelA confers a short, compact spike with fewer spikelets per spike compared to LDN. Molecular mapping and quantitative trait loci (QTL) analysis of these traits in a homozygous recombinant population derived from LDN × the chromosome 2A substitution line (LDNIsA-2A) indicated that the number of spikelets per spike and spike length were controlled by linked, but different, loci on the long arm of 2A. A QTL explaining most of the variation for spike compactness coincided with the QTL for spike length. Comparative mapping indicated that the QTL for number of spikelets per spike overlapped with a previously mapped QTL for Fusarium head blight susceptibility. The genes governing spike length and compactness were not orthologous to either *sog* or *C*, genes known to confer compact spikes in diploid and hexaploid wheat, respectively. Mapping and sequence analysis indicated that the gene governing spike length and compactness derived from wild emmer could be an ortholog of the barley *Cly1*/*Zeo* gene, which research indicates is an *AP2*-like gene pleiotropically affecting cleistogamy, flowering time, and rachis internode length. This work provides researchers with knowledge of new genetic loci and associated markers that may be useful for manipulating spike morphology in durum wheat.

Keywords Wheat · Spike morphology · Compactness · Wild emmer

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J. D. Faris (✉) · Z. Zhang · S. S. Xu
USDA-Agricultural Research Service NPA NCSL, Cereal Crops Research Unit, Red River Valley Agricultural Research Center, 1605 Albrecht BLVD, Fargo, ND 58102-2765, USA
e-mail: Justin.Faris@ars.usda.gov

D. F. Garvin
USDA-Agricultural Research Service, Plant Science Research Unit and Department of Agronomy and Plant Genetics, 411 Borlaug Hall, University of Minnesota, 1991 Upper Buford Circle, St. Paul, MN 55108, USA

Introduction

Wheat is a major source of human sustenance and therefore one of the most important grain crops in the world. Bread wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD genomes), which is hexaploid, is used primarily to make breads, noodles, crackers, cakes, cookies, and other foods containing wheat flour, whereas durum wheat (*T. turgidum* L. $2n = 4x = 28$, AABB genomes), which is tetraploid, is used to make pasta and other semolina-based products. The wheat spike (also known as the ear or head) is one of the most important parts of a wheat plant because it is harbors the reproductive organs, produces the seeds, and holds the grains until harvest.

In bread wheat, there are three major genes that affect gross morphology of the spike: *Q*, *C*, and *S*. The *Q* gene lies on chromosome arm 5AL and pleiotropically influences not only spike length and shape, but also seed

threshability, glume tenacity, rachis fragility, plant height, and spike emergence time (Unrau et al. 1950; Sears 1952, 1954; MacKey 1954; Muramatsu 1963, 1979, 1985, 1986; Kato et al. 1999, 2003; Faris and Gill 2002; Faris et al. 2003, Simons et al. 2006; Zhang et al. 2011). Therefore, *Q* is considered a major domestication gene as well as a regulator of development. The cloning of *Q* revealed that it is a member of the AP2 class of transcription factors known to be involved in plant development and regulation (Simons et al. 2006; Zhang et al. 2011). Because it is required for the free-threshing trait, the *Q* allele is present in essentially all domesticated common and durum wheat varieties.

The *C* gene lies on chromosome 2D (Johnson et al. 2008) and defines a subspecies of hexaploid wheat known as *T. aestivum* ssp. *compactum* (Host) Mac Key, or club wheat, which has a characteristic compact spike due to a dominant *C* allele. The *s* gene on chromosome 3D defines another subspecies known as *T. aestivum* ssp. *sphaerococcum* (Percival) Mac Key, or shot wheat, which is characterized by having round seed, round glumes, and a short dense spike (Sears 1947). Therefore, common wheat (ssp. *aestivum*) has the genotype *QcS*, club wheat (*compactum*) is *QCS*, and shot wheat (*sphaerococcum*) is *Qcs*.

Whereas the genes involved in governing spike morphology in bread wheat have been well studied, fewer studies regarding spike morphology in durum wheat have been conducted. All durum wheat cultivars and varieties harbor the *Q* allele, and none carry the *C* or *S* genes known to govern spike traits in bread wheat because both are on D-genome chromosomes, which durum, being tetraploid with A and B genomes, does not possess. Therefore, variation in spike morphology among durum wheats would presumably be due to genes other than *Q*, *C*, or *S*, or possibly due to homoeoalleles of these genes on the homoeologous chromosomes. Indeed, factors affecting spike compactness have been reported on nearly every chromosome of wheat (Kuspira and Unrau 1957; Sourdille et al. 2000; Jantasuriyarat et al. 2004).

Sears et al. (1957) demonstrated the development of disomic chromosome substitution lines and provided a model for their utility in the identification and genetic analysis of genes governing important traits. Individual chromosome pairs of the four bread wheat varieties Timstein, Hope, Thatcher, and Red Egyptian were substituted for the corresponding homologous pair of chromosomes in the hexaploid wheat landrace Chinese Spring. Therefore, 21 chromosome substitution lines for each of the four donor varieties in the Chinese Spring background were developed, and these lines were used to identify donor chromosomes harboring stem rust resistance genes (Sears et al. 1957). Since then, many have realized the value of individual chromosome substitution lines and have used them to determine the chromosome locations of major genes as well as dissect quantitative trait loci (QTLs) essentially one chromosome at a time.

Dr. Leonard Joppa (USDA-ARS, retired) extended this model to tetraploid wheat by developing a set of D-genome chromosome substitution lines where a pair of chromosomes in the durum wheat variety Langdon (LDN) was replaced by a pair of homoeologous D-genome chromosomes from the hexaploid Chinese Spring (Joppa and Williams 1988; Joppa 1993). Shortly thereafter, Cantrell and Joppa (1991) reported on the use of the tetraploid wild emmer wheat (*T. turgidum* ssp. *dicoccoides* (Körn.) Thell, $2n = 4x = 28$, AABB genomes) accession FA-15-3 (syn. IsraelA) as the donor for a set of chromosome substitution lines in the LDN background (LDNIsA). This set of LDNIsA substitution lines has been widely distributed and used to locate numerous important agronomic genes to individual chromosomes (Joppa and Cantrell 1990; Cantrell and Joppa 1991; Joppa et al. 1991; Joppa 1993; Stack et al. 2002; Singh et al. 2007).

To further exploit the LDNIsA substitution lines, Joppa (1993 and unpublished) developed populations of homozygous recombinant (HR) lines for each chromosome. These populations are extremely useful because they segregate for a single recombinant chromosome in an otherwise homozygous LDN background allowing the genetic analysis, mapping, and dissection of traits in isolation. The LDNIsA HR populations have been used to identify and map genes for traits such as grain protein content (Joppa et al. 1997), yield (Gonzalez-Hernadez et al. 2004), rachis brittleness (Nalam et al. 2006), segregation distortion (Kumar et al. 2007), Fusarium head blight resistance/susceptibility (Otto et al. 2002; Garvin et al. 2009), and *Stagonospora nodorum* blotch and tan spot resistance (Faris et al. 2000; Gonzalez-Hernandez et al. 2009; Faris and Friesen 2009).

General observations of the LDNIsA substitution lines revealed that LDNIsA-2A has a compact spike relative to LDN. The objectives of this work were to (1) use the LDN × LDNIsA-2A HR population to conduct genetic analysis and molecular mapping of the dense spike trait derived from wild emmer; and (2) conduct comparative mapping analysis to determine putative homoeologous and/or orthologous relationships with other Triticeae genes that confer spike compactness.

Materials and methods

Plant materials

The durum wheat variety LDN, the LDNIsA-2A substitution line, and the population of 107 HR lines derived from LDN × LDNIsA-2A and developed by L.R. Joppa were used for mapping spike compactness derived from the *T. turgidum* ssp. *dicoccoides* accession Israel A, which was used as the donor for the LDNIsA-2A chromosome

substitution line. Dr. L.R. Joppa also developed two additional sets of *T. turgidum* ssp. *dicoccoides* chromosome substitution lines in the LDN durum background using the *T. turgidum* ssp. *dicoccoides* accessions PI 478742 and PI 481521 as donors (Klindworth et al. 2009), henceforth designated LDN742 and LDN521, respectively. The substitution line LDN742-2A is not available, but lines LDN742-2B, LDN521-2A, and LDN521-2B were included in this study and evaluated for spike compactness. The LDNIsA-2B line is also available, but it is a weak plant lacking vigor and therefore not included in this study.

In addition to the LDN-*T. turgidum* ssp. *dicoccoides* substitution lines, we also included the *T. turgidum* ssp. *dicoccoides* chromosome 2A and 2B disomic substitution lines in the *T. aestivum* cultivar Chinese Spring (CS) for evaluation of spike compactness. These lines were produced by the late E.R. Sears using the *T. turgidum* ssp. *dicoccoides* accession TA106 as the donor, and will hereafter be referred to as CS106-2A and CS106-2B, respectively. Other materials used in this research include the CS chromosome deletion lines involving chromosome 2A (Endo and Gill 1996) for assigning SSR fragments to chromosome deletion bins. TA106, CS106-2A, CS106-2B, and the chromosome 2A deletion lines were provided by the Wheat Genetics Resource Center, Kansas State University, Manhattan, KS.

Phenotypic evaluation and statistical analysis

Plants were grown in 6-in. clay pots with Metro Mix 902 soil (Hummert International, Earth City, MO) and supplemental fertilizer in a greenhouse at a temperature of 21 °C and a 16/8 h light/dark photoperiod. LDN, LDNIsA-2A, and the 2A HR population were grown in a completely randomized design (CRD) consisting of three replicates with one plant per pot. LDN, CS, and the seven disomic substitution lines mentioned above were grown in a CRD consisting of five replicates with one plant per pot. This experiment was repeated once for a total of ten replicates.

Spikes were harvested at maturity and dried at 32 °C for at least 24 h. Four spikes from each plant were measured from the base of the first spikelet to the tip of the most terminal spikelet excluding the awns, and the number of spikelets for each spike was recorded. The degree of compaction was calculated by dividing the spike length by the number of spikelets. Data for the four spikes from each plant were used to calculate the average scores for each replicate. Bartlett's Chi-squared test was conducted using PROC GLM in SAS program version 9.3 (SAS Institute 2011) to determine homogeneity of error variances among replicates. Because error variances among replicates were homogeneous (data not shown), the values for spike length, number of spikelets, and compactness for each replicate were combined to derive the overall means, which were used for quantitative trait loci (QTL) analysis. Fisher's least significant difference (LSD) test was used to determine significant differences among means of the different genetic stocks and the HR lines at the 0.05 level of probability.

Molecular mapping and QTL analysis

A linkage map of chromosome 2A was previously generated in the LDN × LDNIsA-2A HR population consisting of 12 simple sequence repeat (SSR) markers (Garvin et al. 2009). To enhance the map, we evaluated 35 primer sets from BARC (Song et al. 2005), CFA (Sourdille et al. 2004), HBG (Torada et al. 2006), GWM (Röder et al. 1998), KSUM (Yu et al. 2004), and WMC (Somers et al. 2004) SSR libraries for polymorphism between the parents using the PCR and DNA fragment visualization methods described in Zhang et al. (2009).

The rice gene Os04g55560 was reported to be a homolog of *HvAP2* of barley, which cosegregated with the *Flowering time-2L* (*Flt-2L*) locus (Chen et al. 2009a). The genomic sequence of Os04g55560 was downloaded from the Rice Genome Annotation Project website (http://rice.plantbiology.msu.edu/) and used as a query in BLASTn searches of the NCBI nonredundant database to identify homologous sequences for the *Triticum* A and B genomes. This search revealed several *AP2*-like sequences derived from wheat A and B genomes, which were homologs of the barley cleistogamous (*Cly1*)/zeocritron (*Zeo*) gene (Nair et al. 2010; Houston et al. 2013; Ning et al. 2013). The primers specific for the chromosome 2A copy of the wheat *Cly1/Zeo* homolog, *TaAP2-2A*, reported by Ning et al. (2013) were tested for polymorphism between LDN and LDNIsA-2A, but did not reveal polymorphism. Therefore, the entire sequence of the Chinese Spring BAC containing *TaAP2-2A* (GenBank accession no. AB749308) was downloaded and surveyed for SSRs. Primers designed for an SSR 346 bp downstream from the stop codon of the *TaAP2-2A* gene revealed polymorphism between LDN and LDNIsA-2A and were used to map the *TtAP2-2A* locus. This marker was designated as *Xfcp651(Zeo-2A)* and the primer sequences are 5′-AGAAGACGATCGAGAGAG ATT-3′ and 5′-AAAAGCACCAAAAGATACTCC-3′.

The linkage map for chromosome 2A was assembled using MapDisto 1.7.5.2 (Lorieux 2012) using an LOD threshold for marker linkages of 3.0 and a maximum distance between markers of 30.0 cM. The 'order sequence', 'check inversions', 'ripple order', and 'drop locus' features were employed to determine the best order of markers. The Kosambi mapping function (Kosambi 1944) was used to calculate genetic distances between markers.

Mean values for spike length, number of spikelets per spike, and spike compactness for the 2A HR population

were used for QTL analysis. The software program QGene (Joehanes and Nelson 2008) was used to conduct simple interval mapping (SIM) to identify markers or marker intervals significantly associated with the phenotypic data. Composite interval mapping was not used because the population segregates for only the 2A chromosome. A test consisting of 1,000 permutations indicated that a critical LOD threshold of 2.0 represents an experiment-wise significance level of 0.05.

Sequencing and sequence analysis

The chromosome 2A orthologs of the barley *Cly1/Zeo* gene (Ning et al. 2013; Houston et al. 2013) in CS, CS106-2A, LDN, LDNIsA-2A, and LDN521-2A were PCR-amplified using the primers and conditions described in Ning et al. (2013). PCR amplicons were electrophoresed on 1.5 % agarose gels, gel-purified using the Wizard® SV Gel and PCR Clean Up System from Promega (Madison, WI), and sequenced using the Sanger method by Eurofins MWG Operon (Huntsville, AL). Genomic and deduced amino acid sequences were aligned using MacVector 11.0.2. The *Cly1/Zeo* AP2-like genes sequenced from chromosome 2A of CS106-2A, LDN, LDNIsA-2A, and LDN521-2A have the GenBank accession numbers KJ125393–KJ125396, respectively. The gene from CS was previously submitted to GenBank and has the accession number AB749311 (Ning et al. 2013).

Results

Phenotypic evaluations

As mentioned above, general observation of LDN and LDNIsA-2A indicated that the latter had a shorter and more compact spike relative to the former. Detailed analysis of spikes of these lines showed that indeed, the average spike length of LDNIsA-2A was more than 2 cm shorter (68 %) than that of LDN (Table 1; Fig. 1). LDNIsA-2A also had four fewer spikelets than LDN on average. Despite this, LDNIsA-2A spikes showed significantly increased compactness relative to LDN spikes.

The other *T. turgidum* ssp. *dicoccoides* chromosome 2A substitution in the LDN background, LDN521-2A, did not differ significantly from LDN in spike length, number of spikelets per spike, or compactness (Table 1; Fig. 2). Neither of the two LDN substitution lines involving chromosome 2B from *T. turgidum* ssp. *dicoccoides* accessions PI 478742 and PI 481521 had spike lengths that differed significantly from that of LDN (Table 1; Fig. 2). However, both LDN742-2B and LDN521-2B had significantly fewer spikelets per spike compared to LDN, although not as few as the LDNIsA-2A substitution line. Having the same length of spike but fewer spikelets resulted in LDN742-2B and LDN521-2B having less compacted spikes compared to LDN. Both chromosome 2B substitution lines had non-glaucous spikes compared to the glaucousness of LDN. This was likely due to the presence of the glaucousness inhibitor gene *Iw2* on 2BS (Tsunewaki 1966; Tsunewaki and Ebona 1999) of the *T. turgidum* ssp. *dicoccoides* accessions.

Table 1 Spike length, number of spikelets per spike, and spike compaction in Chinese Spring, Langdon, and disomic chromosome substitution lines involving chromosomes 2A and 2B from *Triticum turgidum* ssp. *dicoccoides*

Line	Spike length (cm)[a]	No. spikelets	Compactness[b]
Langdon	6.90a	22.46b	0.31c
LDNIsA-2A	4.69b	18.25e	0.25d
LDN742-2B	7.23a	20.17d	0.36a
LDN521-2A	6.61a	21.91bc	0.30c
LDN521-2B	7.12a	20.68 cd	0.35ab
Chinese Spring	7.36a	24.35a	0.30c
CS106-2A	5.86c	21.53bcd	0.28 cd
CS106-2B	7.20a	23.65ab	0.31bc

[a] Numbers followed by the same letter within the same column are not significantly different at the 0.05 level of probability as determined by least significant difference (LSD)

[b] Compactness was calculated by dividing the spike length by the number of spikelets

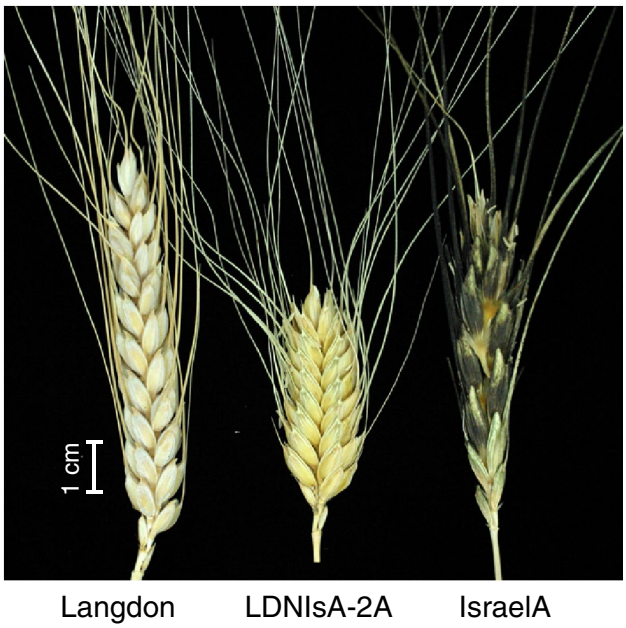

Fig. 1 Mature spikes of Langdon, the Langdon-*Triticum turgidum* ssp. *dicoccoides* accession IsraelA chromosome 2A substitution line (LDNIsA-2A), and *T. turgidum* ssp. *dicoccoides* accession IsraelA

Fig. 2 Mature spikes of Langdon, the Langdon-*Triticum turgidum* ssp. *dicoccoides* accession PI481521 chromosomes 2A and 2B substitution lines (LDN521-2A and LDN521-2B), the Langdon-*T. turgidum* ssp. *dicoccoides* accession PI478742 chromosome 2B substitution line (LDN742-2B), Chinese Spring, and the Chinese Spring-*T. turgidum* ssp. *dicoccoides* accession TA106 chromosomes 2A and 2B substitution lines (CS106-2A and CS106-2B)

Compared to Chinese Spring, the CS106-2A substitution line had spikes that were about 1.5 cm shorter (Table 1; Fig. 2). But because CS106-2A also had three fewer spikelets per spike, Chinese Spring and CS106-2A did not differ in spike compactness. The *T. turgidum* ssp. *dicoccoides* chromosome 2B substitution line CS106-2B did not differ from Chinese Spring for any of the traits.

The population of HR lines derived from LDN × LDNIsA-2A segregated for spike length, spikelets per spike, and spike compactness. Average spike lengths ranged from 4.3 to 7.5 cm with a mean of 6.2 cm (Fig. 3). The number of spikelets per spike among the HR lines ranged from an average of 14.5 to 24.3 spikelets per spike with a mean of 20.8, and compactness scores ranged from 0.24 to 0.42 with a mean of 0.30 (Fig. 3).

Based on statistically significant differences ($P < 0.05$), only a few transgressive segregants were observed for the three spike traits evaluated. For spike length, one HR line was significantly shorter than LDNIsA-2A and no HR lines had longer spikes than LDN. Two HR lines had fewer spikelets per spike compared to LDNIsA-2A, but none had more spikelets per spike than LDN. For compactness, no HR line had a more compact spike than LDNIsA-2A, but three HR lines were significantly less compact than LDN.

Molecular mapping

We added ten SSR markers to the map of chromosome 2A generated by Garvin et al. (2009) (Fig. 4). The addition of these markers extended the map length from 157.0 to 199.1 cM. Most of the additional length was due to six additional markers placed at the distal end of the long arm.

Data for spike length, number of spikelets per spike, and spike compactness from the means of the three replicates were regressed on the genotypic data for chromosome 2A markers using simple interval regression mapping. Interval mapping indicated that a QTL flanked by markers *Xwmc181* and *Xfcp651(Zeo-2A)* was significantly associated with spike length (Fig. 4). The LOD for this QTL, designated *QEl.fcu-2A*, was 17.3 and it explained 52.6 % of the phenotypic variation (Table 2; Fig. 4). A QTL for number of spikelets per spike was associated with the marker *Xgwm445*, which mapped proximal to *QEl.fcu-2A* (Table 2; Fig. 4). This QTL, designated *QSpn.fcu-2A*, explained 19.4 % of the variation and had a LOD value of 5.0. A QTL for spike compactness designated *QCmp.fcu-2A* co-located with the QTL for spike length flanked by *Xwmc181* and *Xfcp651(Zeo-2A)* (Table 2; Fig. 4). *QCmp.fcu-2A* had a LOD value of 18.2 and explained 54.3 % of the variation.

Deletion-based physical mapping

Markers *Xwmc181* and *Xfcp651(Zeo-2A)* were not previously placed on the 2A deletion-based physical map (Sourdille et al. 2004). *Xwmc181* was found to map within the bin flanked by breakpoints in 2AL-3 and 2AL-1, whereas *Xfcp651(Zeo-2A)* mapped in the bin distal to 2AL-1 (Fig. 4). *Xgwm445*, the marker most closely associated with the QTL for spikelet number per spike (*QSpn.fcu-2A*), was shown by Röder et al. (1998) to map proximal to the 2AL-3 breakpoint. These results indicate that the gene

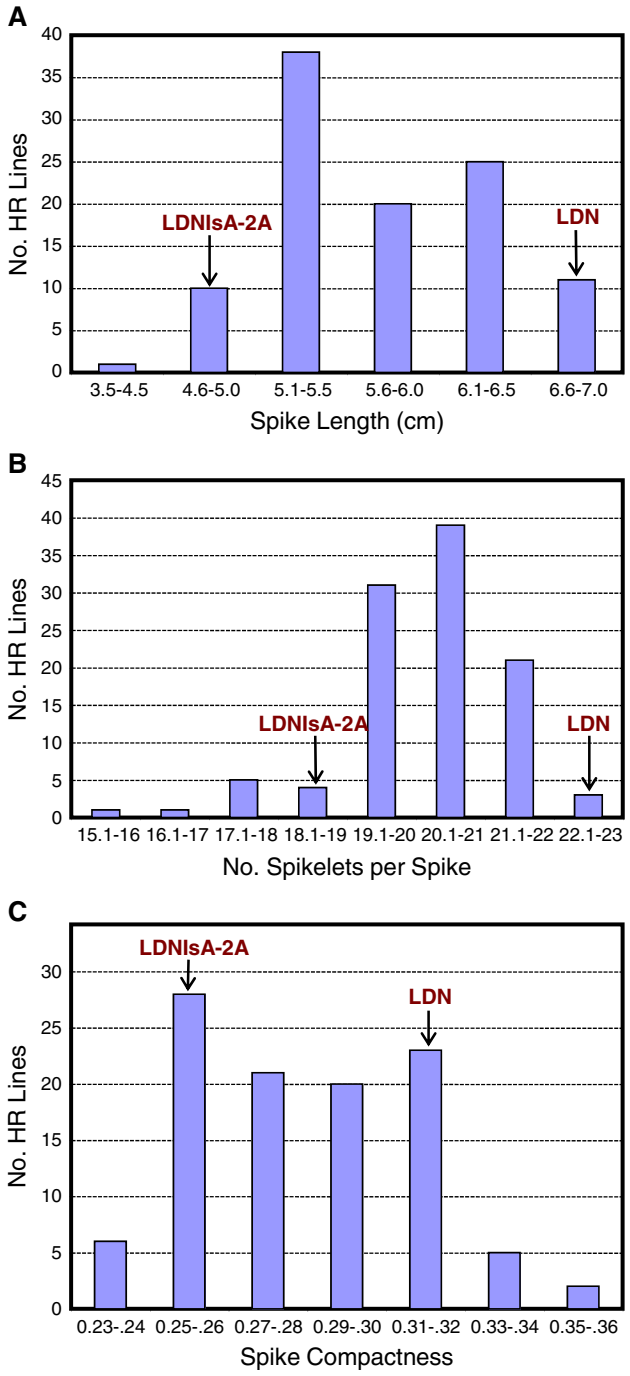

Fig. 3 Histograms for spike length (**a**), number of spikelets per spike (**b**), and spike compactness (**c**) in the LDN × LDNIsA-2A HR population

governing the effects of *QSpn.fcu-2A* lies between the centromere and the breakpoint in 2AL-1, but it could not be determined which side of the 2AL-3 breakpoint the gene resides. Similarly, the gene(s) governing the spike length and compactness QTLs is distal to 2AL-3, but it could not be determined whether it lies proximal or distal to the 2AL-1 breakpoint.

Map comparisons

The physical map locations of the spike trait QTLs derived from wild emmer wheat were compared to those of other spike morphology genes to determine if they might be orthologous or homoeologous. The other genes included *sog*, which confers soft glumes and a dense spike, and lies on chromosome $2A^m$ of *T. monococcum* (Sood et al. 2009); the *C* gene of *T. compactum*, which confers a very dense "club" spike and maps to chromosome 2D (Johnson et al. 2008); and the wheat ortholog of the barley *Cly1/Zeo* gene, which governs cleistogamy (Nair et al. 2010), spike density (Houston et al. 2013), and affects flowering time, rachis internode length, and plant height (Chen et al. 2009b). The *sog* locus of *T. monococcum* was shown by Sood et al. (2009) to be located on the short arm of $2A^m$ between the centromere and a breakpoint represented by 2DS-5 on the physical map (Fig. 4). Johnson et al. (2008) were unable to precisely determine the physical location of the *C* gene, but narrowed it to the centromeric region delineated by breakpoints in deletion lines 2DS-1 and 2BL-4. More recent work indicates that *C* is located on the short arm of 2D (V. Tiwari, personal communication). Therefore, the wild emmer QTLs for spike length, spikelet number, and compactness are not orthologous with *C* or *sog*. However, the location of the wheat *Cly1/Zeo* homolog distal to the 2AL-1 breakpoint (Ning et al. 2013) allows for the possibility that the *APETELA2*-like gene could be responsible for the effects of QTLs *QEl.fcu-2A* and *QCmp.fcu-2A* from wild emmer.

Comparative sequence analysis

Because the wheat chromosome 2A ortholog of the barley *Cly1/Zeo* gene was considered a candidate for the spike length and compactness QTLs, we sequenced the gene from CS, CS106-2A, LDN, LDNIsA-2A, and LDN521-2A. Deduced amino acid alignments indicated that the sequences were highly conserved and no differences existed within the putative *miR172* microRNA binding site (Fig. 5), which was shown to be responsible for governing phenotypic differences in barley (Houston et al. 2013). However, CS harbored two deletions consisting of three amino acids each at positions 327–329 and 403–405 compared to CS106-2A. Also, LDN harbored a seven-amino acid deletion at position 306–312 compared to LDNIsA-2A, which was identical to CS106-2A. LDN521-2A had a two-amino acid deletion (position 404–405) compared to LDNIsA-2A.

Discussion

Spike compactness is actually a function of two traits (spike length and number of spikelets) potentially controlled

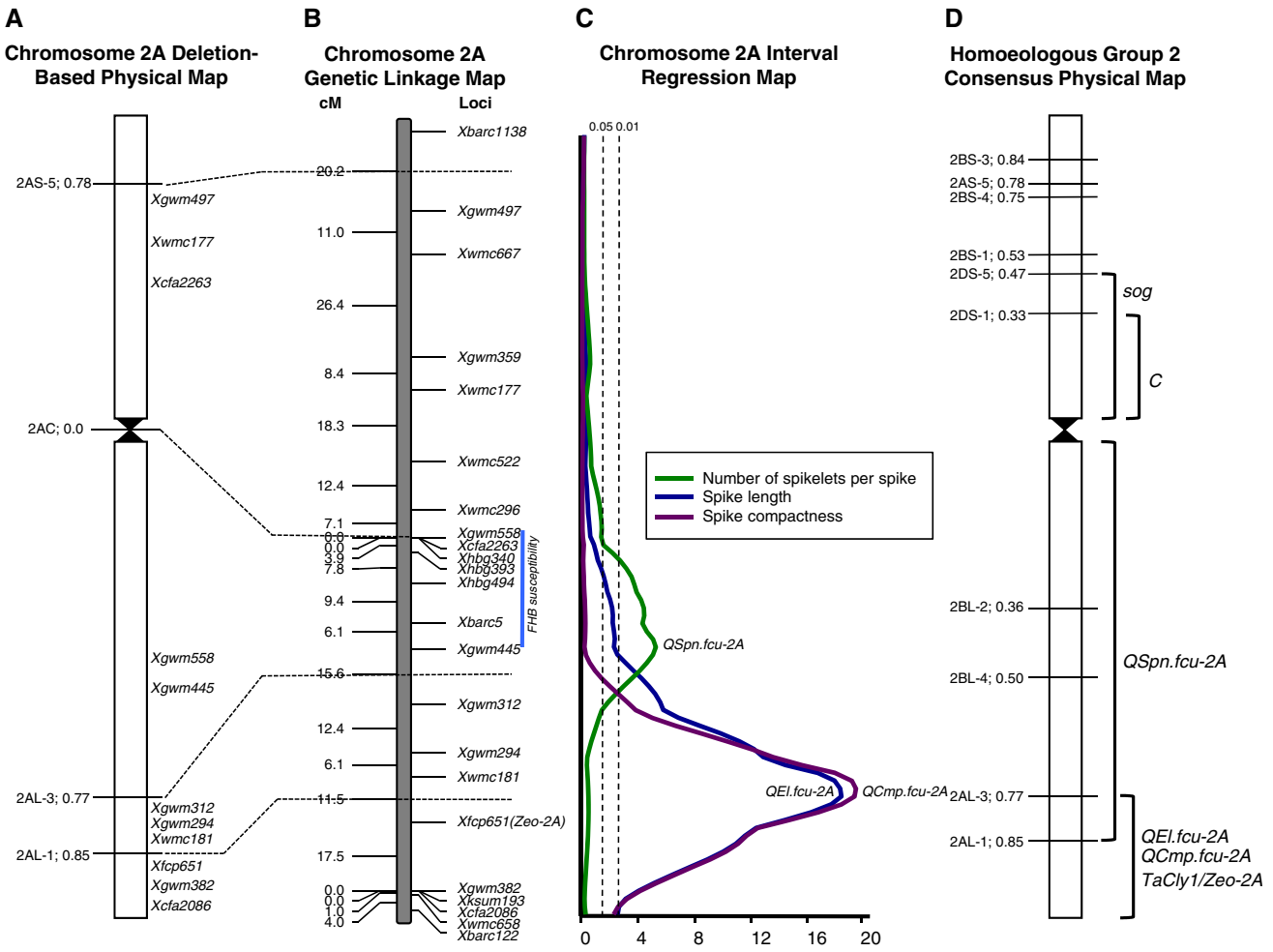

Fig. 4 Maps of wheat chromosome 2A. **a** Chromosome 2A deletion-based physical map. Fraction breakpoints are indicated along the *left side* and markers are placed in their bin locations along the *right*. **b** The chromosome 2A genetic linkage map developed in the LDN × LDNIsA-2A HR population. Genetic distances in centiMorgans (cM) are shown along the *left* and markers are shown along the *right*. The location of the FHB susceptibility QTL reported by Garvin et al. (2009) is indicated by the *blue vertical line*. *Dotted lines* between **a** and **b** are to indicate the approximate locations of the deletion breakpoints on the genetic linkage map. **c** An interval regression map developed by conducting simple interval mapping of the average number of spikelets per spike (*green line*), average spike length (*blue line*), and average spike compactness (*purple line*) in the LDN × LDNIsA-2A HR population. The approximate peaks of the QTL regression lines are indicated by the QTL designations. The 0.05 and 0.01 significance thresholds are indicated by the *vertical dotted lines*, and an LOD scale is indicated on the *x* axis. **d** A consensus physical map of wheat homoeologous group 2 chromosomes showing the approximate locations of deletion breakpoints along the left and known bin locations of the *T. monococcum sog* gene (Sood et al. 2009), the *T. aestivum* ssp. *compactum C* gene (Johnson et al. 2008), the *TaCly1/Zeo* gene (Ning et al. 2013), and the QTLs *QSpn.fcu-2A*, *QEl.fcu-2A,* and *QCmp.fcu-2A* identified in this research (color figure online)

Table 2 QTLs associated with number of spikelets per spike, spike length, and spike compactness detected by simple interval mapping

Trait	QTL	Chrom. arm	Marker interval	LOD	R^2
No. spikelets per spike	*QSpn.fcu-2A*	2AL	*Xbarc5-Xgwm445*	5.0	0.19
Spike length	*QEl.fcu-2A*	2AL	*Xwmc181-Xfcp651(Zeo)*	18.6	0.55
Spike compactness	*QCmp.fcu-2A*	2AL	*Xwmc181-Xfcp651(Zeo)*	19.0	0.56

The chromosome arm locations, associated markers, LOD, and R^2 values are given

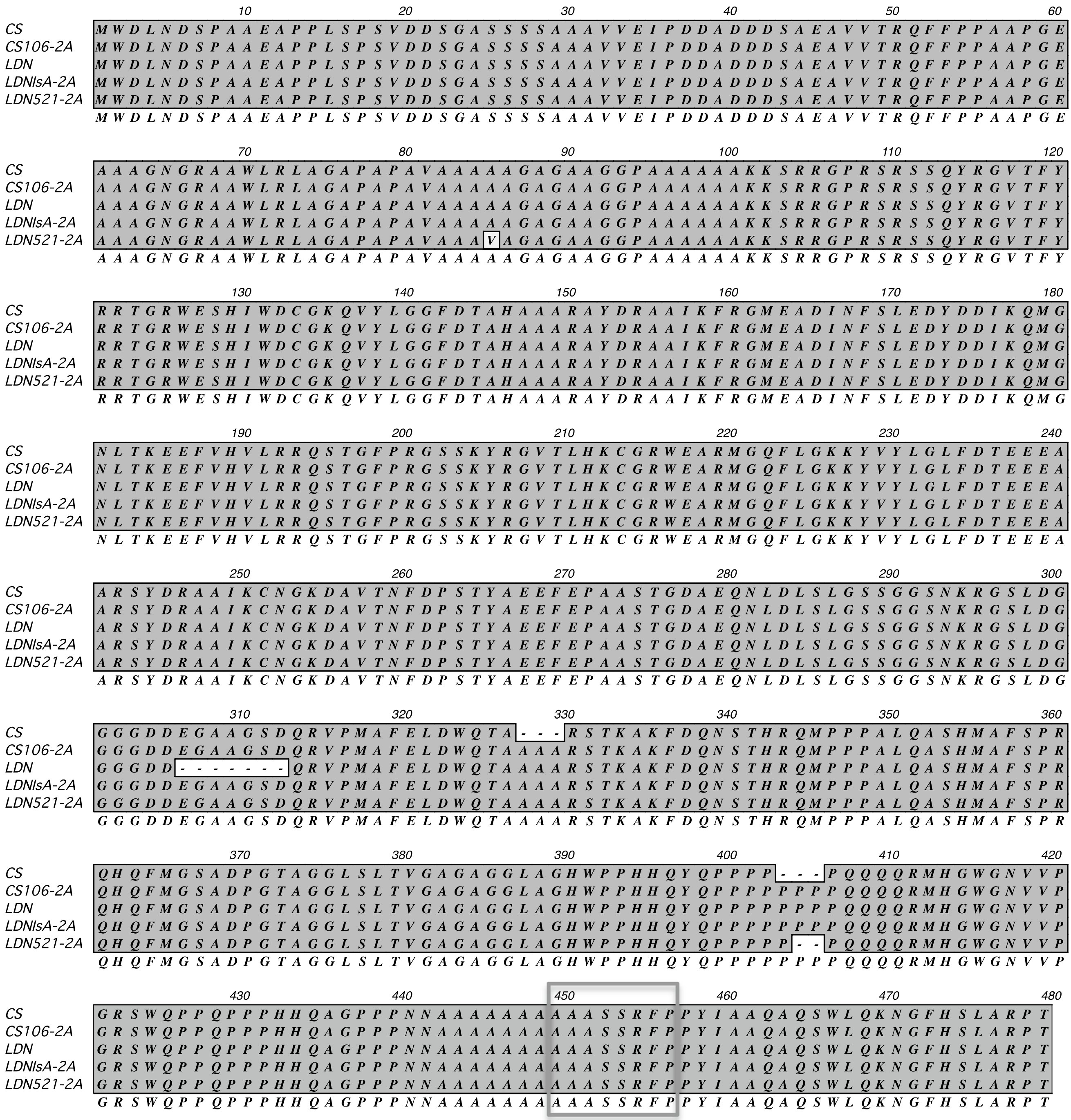

Fig. 5 Deduced amino acid sequence alignment of the Cly1/Zeo AP2-like ortholog from chromosome 2A of the genetic stocks CS, CS106-2A, LDN, LDNIsA-2A, and LDN521-2A. The putative miRNA 172 binding site is indicated by the *grey box*

by multiple genes. In this research, we identified different QTLs on the long arm of 2A governing the number of spikelets per spike and spike length. A shortened spike and fewer spikelets were traits contributed by LDNIsA-2A, whereas a longer spike and more spikelets per spike were contributed by LDN. The relatively minor QTL *QSpn.fcu-2A* primarily affected the number of spikelets per spike, but was also significantly (0.05 level of probability) associated with spike length. This is expected because, by definition, any factor affecting spikelet number per spike must also be associated with spike length and/or compactness. On the contrary, the relatively major QTL *QEl.fcu-2A* affected spike length and spike compactness, but not spikelet number per spike.

Whereas more than 50 % of the phenotypic variation for spike length was explained by *QEl.fcu-2A*, <20 % of the variation in spikelet number per spike was explained by *QSpn.fcu-2A* indicating that the latter is more influenced by environmental factors, experimental error, or other undetected genetic factors that may have contributed to the relatively small magnitude of the QTL. Also, the spike compactness QTL (*QCmp.fcu-2A*) coincided nearly perfectly with the spike length QTL (*QEl.fcu-2A*) (Fig. 4), which reflects the strong influence of the spike length variable in the spike compactness equation.

Other studies on wheat spike morphology and compactness have revealed that, like the results of our study, compactness QTLs usually coincide with QTLs for spike length as opposed to the number of spikelets per spike. For example, Sourdille et al. (2000) evaluated a hexaploid wheat population and reported QTLs for spike length on 1A, 2B, 2D, 4A, and 5A, all of which coincided with QTLs for spike compactness. All QTLs associated with spike length were also associated with spike compactness. However, of three QTLs associated with spikelet number per spike, two on chromosomes 2A and 5A did not coincide with compactness QTLs. Only a QTL on the short arm of 2B was associated with all three traits; spikelet number per spike, spike length, and spike compactness. In addition, none of the QTLs identified by Sourdille et al. (2000) on homoeologous group 2 chromosomes were homologous or homoeologous to the QTLs reported here, and were instead located on the short arms and suggested to be governed primarily by the photoperiod response (*Ppd*) loci.

Using a different hexaploid wheat population, Jantasuriyarat et al. (2004) reported spike length QTLs that coincided with spike compactness QTLs on 1B and 6A, a spikelet number per spike QTL on 7A, and coinciding QTLs on 4A for spike length, spikelet number per spike and spike compactness. Together, the results of these studies and the current one indicate that spike length and spikelet number per spike are largely controlled by different genes and that spike compactness QTLs, despite being a function of spikelets per spike and spike length, usually coincide with spike length QTLs.

Fusarium head blight (FHB) is a devastating fungal disease of durum and common wheat. The fungus infects the spikes causing losses in yield and grain quality due to shriveled, scabby kernels and the production of mycotoxins. A QTL conferring FHB susceptibility derived from LDNIsA-2A was previously identified using the LDN × LDNIsA-2A HR population (Garvin et al. 2009). Comparison of the location of that QTL with those identified in this research indicates that the spike length/compactness QTLs are located more distal on 2AL (Fig. 4) and are probably not responsible for the effects of the FHB susceptibility QTL reported by Garvin et al. (2009). However, the confidence intervals for the FHB susceptibility QTL and the QTL for spikelet number per spike (*QSpn.fcu-2A*) do overlap making it possible that they are governed by the same underlying gene. This may be considered unlikely because a narrow flower opening width, likely associated with compact spikes, has been shown to be associated with resistance to FHB (Gilsinger et al. 2005) rather than susceptibility.

The barley *Cly1* gene confers the cleistogamous phenotype, which is the failure of the lodicules to swell, thus the lemma and palea are not forced apart until well after fertilization (Nair et al. 2010). The *Cly1* gene has been cloned and shown to be a member of the *AP2* class of transcription factors residing on the long arm of barley chromosome 2H (Nair et al. 2010). Earlier, Chen et al. (2009a) reported the barley 2HL *AP2*-like gene to be a strong candidate for the flowering time locus *Flt-2L*. This locus was shown to influence not only flowering time, but also spike length and cleistogamy as well (Chen et al. 2009b). Therefore, the barley *Cly* and *Flt-2L* genes are likely the same with their identity being the chromosome 2HL *AP2*-like gene. In addition to this, the barley *Zeo* gene, which confers a dense spike in barley (Druka et al. 2011), was recently isolated and shown to also be an *AP2*-like gene on 2HL, and the same gene as *Cly1* (Houston et al. 2013). The ortholog of this barley *AP2*-like gene on wild emmer chromosome 2A is a potential candidate for the spike length/compactness QTLs identified in this research. The 2A wheat ortholog represented by marker *Xfcp651(Zeo-2A)*, mapped near the peak of the spike length and compactness QTLs. Although sequence analysis revealed no differences within the miR172 binding site of the chromosome 2A *Cly1/Zeo* orthologs of wheat, as was shown to be the case for governing phenotypic differences in barley, other differences were identified that could potentially alter function.

The *Cly1/Zeo AP2*-like genes of CS, LDN, and LDN521-2A all had deletions that resulted in a lack of amino acids compared to CS106-2A and LDNIsA-2A, which had amino acid sequences identical to each other. CS, LDN, and LDN521-2A did not differ significantly in spike length or compactness, whereas CS106-2A and LDNIsA-2A had spikes that were significantly shorter than LDN and CS. It is possible that a 'reduction' or 'abolishment' of function due to the deletions in the CS, LDN, and LDN521-2A copies confers a longer, more lax, spike compared to when the deletions are not present. It is worthy to note that the wheat *Q* gene, which is also an *AP2*-like gene but on chromosome arm 5AL, confers a relatively dense spike in its wild-type form (Muramatsu 1963) and a severely compact spike when overexpressed, but a much longer, more lax spike when it is mutated or harbors deletions (Simons et al. 2006). Therefore, it is possible that the chromosome 2A *Cly1/Zeo* ortholog is responsible for the dense spike trait of LDNIsA-2A and CS106-2A, but more investigation is needed to be certain. Evaluation of the gene in more durum and/or bread

wheat varieties to determine if deletions are consistently present, mutagenesis, and/or transformation experiments would ultimately provide proof.

Comparisons among the LDN-*T. turgidum* ssp. *dicoccoides* 2B and 2A chromosome substitution lines revealed that, whereas substitution of chromosome 2A from the accession IsraelA into the LDN background resulted in a shorter, more compact spike with fewer spikelets, chromosome 2A from accession PI481521 did not alter these traits. This indicates that the genes conferring a relatively shorter, more compact spike with fewer spikelets are not universal among wild emmer wheats accessions. It is noteworthy, however, that Peng et al. (2003) reported a gene influencing spikelet number per spike on 2AL in the wild emmer accession Hermon H52, which may be the same as *QSpn.fcu-2A* identified in this research.

The dense spike phenotype is not expressed in the wild emmer accession IsraelA, from which it is derived. Only when the 2A chromosome from IsraelA is substituted for the native LDN 2A chromosome in the LDN background are the effects on spike morphology obvious. This suggests that the expression of spike compactness in the IsraelA background is likely suppressed by other genetic factors, which is a phenomenon reminiscent of the FHB resistance QTL identified on IsraelA chromosome 3A (Otto et al. 2002). The 3A QTL *QFhs.ndsu-3A* was not expressed in the IsraelA background, possibly due to the presence of the FHB susceptibility QTL on 2A (Garvin et al. 2009), but was found to confer a high level of FHB resistance in the LDNIsA-3A chromosome substitution line (Stack et al. 2002). These findings demonstrate the value of the disomic chromosome substitution lines for trait discovery and genetic studies.

Triticum aestivum ssp. *compactum* cultivars, or club wheats, tend to be somewhat drought tolerant and resistant to seed shattering presumably due to the compact spike morphology. The compact spike of LDNIsa-2A is more difficult to thresh than the more lax LDN spike (data not shown). Therefore, from a practical standpoint, it is possible that the compact spike trait from LDNIsA-2A could be useful for the development of seed shatter-resistant varieties so long as yield is not affected. More research is needed to test the plausibility of this notion.

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